UPON THE OCCURRENCE OF HAEMOSPORIDIA IN THE BLOOD OF RANA CATESBIANA, WITH AN ACCOUNT OF THEIR PROBABLE LIFE HISTORY

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WITH TWO PLATES

Twenty-four small bullfrogs captured last fall on Long Island, N. Y., furnished the material for this investigation. Upon examination these frogs were found to be quite heavily infected with haemosporidia, while in several of the number trypanosoma were also found. The blood was examined in the fresh and stained conditions, and several forms of parasites were found to be present, which for convenience of reference will temporarily be referred to by number.

One of the parasites which we will designate as No. 5 differs so considerably from any of the others that I consider it to be a distinct species, and will therefore exclude this one, and confine my remarks to a description of the other four forms which will be shown to belong to one and the same species, only in different stages of their life history.

Parasite No. 1.—This parasite was found very plentifully in the red blood corpuscles of the frogs examined, but also is occasionally found in the leucocytes. It is a small gregarine-like organism, somewhat crescent-shaped; pointed at both ends, though at times some may be found pointed at one extremity, and slightly enlarged, and rounded at the other.

It is provided with a nucleus, nearly centrally located, consisting of chromatin granules arranged either in the shape of a ring, irregularly in clumps, or scattered over the body of the parasite. The body cytoplasm contains numerous fine chromatoid granulations, and occasionally the parasite may be found with a vacuole on either side of the nucleus. The length is 18.5 μ, and the diameter 4.6 μ.

The organism stains faintly with the Wright stain, but, strongly with the Goldhorn polychrommethylene blue and eosin stain, the latter staining the body protoplasm a light pink, and the chromatin granules a strong and fiery red.

This parasite (Pl. VII, fig. 2) which I have christened *Haemogregarina catesbianae,* and which later will be shown to be the asexual organism, exerts but little if any action upon the invaded erythrocytes. Occasionally a red cell may be found whose nucleus has been displaced to one side, but as a rule they are not displaced, nor have I ever noticed any other injurious action upon the same. There may be multiple infection with one, two, or three parasites. The organism may be found within the red corpuscles completely elongated, curved up crescent-fashion, with the ends folded up U-shape, or rolled up into a spherical form, depending upon what stage of the asexual cycle it has entered.

Parasite No. 2, or Cytocyst.—(Plate VII, figs. 5 and 8.) Strictly speaking, this is not a distinct or separate parasite, but merely one of the transformation forms of the previously described organism, and represents the encystation stage of the same. This cyst, properly speaking cytocyst, varies considerably in shape and size, though the spherical shape seems to predominate, but it is sometimes found of an ovoid shape. It is surrounded with a thick membrane, which stains of a deep purplish brown color with the Goldhorn stain, while the body of the cytocyst takes a light pinkish shade.

This cytocyst, in reality a schizont, during the later stages of its growth, will be found to contain numerous small merozoites, surrounding a small amount of residual protoplasm, which stain of a fiery red color with the Goldhorn stain (Pl. VII, figs. 5 and 8). The erythrocytes are subject to multiple infection, as many as four schizonts in one corpuscle having been observed. The nuclei of the invaded red cells are usually displaced a little to one side in order to make room for the schizont, but they may frequently also be seen with the nuclei in their normal position. Apart from this displacement of the cell nucleus, no other pathological condition was observed. The diameter of this form is 7.18 μ.

Merozoites.—(Pl. VII, fig. 1.) In addition to the foregoing, a very small ovoid to spherical organism with a fiery red chromatin granule centrally located, was frequently encountered in a free state in the blood plasma, and was usually found quite close to the peri-

phery of a red corpuscle. These bodies are the merozoites, which have escaped from the before-mentioned cytocysts after segmentation.

Parasite No. 3, or Microgametocyte.—(Plate VIII, figs. 1–3.) This parasite is found free in the blood plasma. It is a straight or somewhat curved gregarine-like organism, sharply pointed at one end, but, somewhat more rounded at the other, or anterior end. It swims with its blunter anterior end forward. The average length is 14.45 μ and the diameter is 3.98 μ. The body cytoplasm is slightly granular, and stains of a pale pink color with the Goldhorn stain. The parasite may be found with a centrally located nucleus, consisting of a conglomeration of chromatin granules, as in Pl. VIII, figs. 1 and 2, or with the latter scattered throughout the body of the parasite, as in Pl. VIII, fig. 3. It is very motile, and glides through the blood plasma quite rapidly with an even undulating motion. It is also capable of exerting considerable force, sufficient to easily push any blood corpuscles aside which may happen to be in its path.

One of the most striking peculiarities of this parasite is the ease with which it is able to enter and leave the blood corpuscles. By carefully watching one of these organisms, it may be seen to glide up to a red blood cell with its rounded end first, and immediately proceed to penetrate it, and this is achieved so rapidly that up to the present time I have been unable to discover how it is accomplished. All that can be observed is a slight indentation of the cell protoplasm at the point of contact, when the latter seems to yield to the pressure exerted by the parasite, and before one can realize it, the vermicule has completely buried itself within the corpuscle, leaving as a rule no other sign of its presence within other than a slight writhing motion, and distortion of the cell protoplasm, but at other times its movements may be easily followed. After a varying length of time the parasite will leave its temporary abode, and when this is about to occur, a protuberance will be formed upon the side of the corpuscle from which it is going to emerge. This protuberance gradually increases in length until the erythrocyte is drawn out, pear-shaped. By this time the parasite has practically emerged from the cell, but is still connected with the same by a long, very fine, and nearly invisible thread, with which it tows the corpuscle around for some distance before it is eventually ruptured. It not infre-

quently happens that the parasite when emerging from the blood corpuscle, tears away portions of the same, which it may carry around with it for some time attached to the before-mentioned hyaline thread. This parasite I take to be the microgametocyte of *Haemogregarina catesbianae.* (Pl. VIII, figs. 1, 2 and 3.)

Parasite No. 4, or Macrogametocyte.—(Pl. VIII, figs. 4 and 5.) This parasite exists both in the free state in the plasma, and within the red blood corpuscles. Its length is 9.98μ and its diameter 5.06μ. In the free state it is usually bean-shaped, fairly pointed at one end, but bluntly rounded at the other. Its cytoplasm is quite coarsely and heavily granular, with a well defined nucleus centrally located, or nearly so, and a vacuole on either side of the same, about midway between the nucleus and each pole.

The parasite takes the Goldhorn stain very readily, its cytoplasm staining of a light pink, and the chromatin of the nucleus of a fiery red color. It is not nearly as motile as the microgametocyte. This organism I believe to be the macrogametocyte of *Haemogregarina catesbianae.*

The intra-corpuscular parasite is met with in several forms, depending upon which stage of its life-cycle it has entered. (Pl. VIII, figs. 6–10.) It may be either bean-shaped, ovoid, or spherical, the latter form representing its encystation stage. The nucleus of the intra-corpuscular parasite, or macrogametocyte, is considerably larger than that of the extra-corpuscular organism. In the undivided state it occupies the greater part of the body of the parasite, and stains of a deep fiery red color, with the Goldhorn stain. At times a small vacuole may be found at either pole, but this is not of common occurrence. The cysts, in reality oocysts, are mostly spherical, but vary occasionally, and are sometimes seen of an ovoid shape. They are surrounded by a dense, heavy membrane, which stains of a deep purplish red color. (Pl. VIII, fig. 10.)

It has been an undecided question for some time, how cold-blooded animals like frogs, turtles, snakes, etc., are infected with haemosporidia, some taking the ground that infection is caused by the bite of a blood-sucking insect of some sort, while others believe that infection is induced by taking the parasites through their food into the digestive tract, and from there into the blood.

From my own observation, I know that the latter mode of infection is possible, as will be seen from what is to follow, though this

by no means excludes the other mode of infection, but personally I have been unable to discover any blood-sucking insects or animals likely to carry infection.

A giant bullfrog (*Rana catesbiana*) which I had under observation for over a year, and which was known to be absolutely free from infection of any kind, one day swallowed a small infected bullfrog, which I had carelessly placed in the same aquarium. In about six weeks the large bullfrog's blood was examined, and then found to be infected with the same parasites as discovered in the blood of the small frog. This I think is fairly good evidence that infection may take place through the digestive tract, by means of the food ingested.

An attempt will now be made to show in what is to follow, that both schizogony, and sporogony take place within the blood corpuscles of the same host, though it has usually been assumed that in cold-blooded animals, like the frog, etc., schizogony takes place within the blood corpuscles, while sporogony occurs in the epithelial cells of either the stomach, intestine, liver, or spleen of the same host. In advancing my present views upon this subject, I feel that I am treading upon dangerous ground; nevertheless, as all my observations point in this one direction, I believe that I am justified in the following remarks:

Asexual Cycle of *Haemogregarina catesbianae*.—The cytocyst after segmentation (Pl. VII, fig. 6) discharges its merozoites or spores into the blood plasma, and these after wandering around attach themselves to the blood corpuscles which by some means they manage to penetrate. As soon as this has occurred, the young organism begins to grow, and is converted into a small worm-shaped trophozoite, or schizont (Pl. VII, fig. 7) and this process is continued until the schizont has reached its full growth, when it will have the characteristics previously alluded to. (Pl. VII, fig. 2.) It now begins to fold over on its self, gradually assuming a U-shape. (Pl. VII, figs. 3 and 4.) The two loops of the U now begin to curve inwardly until they meet and coalesce, thus forming a sphere, in which the line of suture is at first visible, but which eventually disappears.

The schizont now surrounds itself with quite a heavy membrane forming a true cyst (Pl. VII, figs. 5 and 8), and the chromatin granules of the fragmented nucleus in turn become surrounded with

a small body of protoplasm, at the expense of the cyst protoplasm, thus forming the merozoites or spores lying about a small amount of residual protoplasm. These after reaching full maturity, rupture the cytocyst, and escape into the plasma, when they are once more ready to invade fresh blood corpuscles. The foregoing represents fairly accurately I believe the asexual cycle of *Haemogregarina catesbianae,* which closely resembles the conditions obtaining with *Lankesterella ranarum,* discovered in the blood of *Rana esculenta,*[1] and whose life history has been described in full by Hintze,[2] in 1902.

Sexual Cycle of *Haemogregarina catesbianae.*—By analogy with the haemosporidia of other cold-blooded animals on the one hand, and with the acystosporidia on the other, it is believed that after many generations of schizogony, the sexes become differentiated into micro- and macrogametocytes, and that sporogony takes place somewhat as follows:

The nucleus of the motile, extra-corpuscular parasite, or microgametocyte (Pl. VIII, figs. 1 and 2), contains a number of chromatin granules. In the course of time the nucleus becomes fragmented, and its chromatin granules divide, and become scattered throughout the body of the parasite. (Pl. VIII, fig. 3.) According to Hintze (*loc. cit.*) the chromatin granules of the fragmented nucleus now become the nuclei of microgametes, which are not separated off simultaneously, but one by one in an irregular manner. This I have been unable to verify in the case of *Haemogregarina catesbianae,* though I suspect that some mode of fertilization must exist.

The unfertilized macrogametocyte which is found free in the blood plasma (Pl. VIII, figs. 4 and 5) is likewise supplied with a nucleus containing a number of chromatin granules. After fertilization, whether this occurs in the manner suggested by Hintze (*loc. cit.*) or by some other mode of conjugation, the macrogametocyte enters a red blood corpuscle, and then prepares for its final encystation, by undergoing a number of changes. It first becomes more ovoid in shape, while the nucleus at the same time is considerably enlarged. (Pl. VIII, fig. 6.) It now begins to divide, or segment (Pl. VIII, fig. 7), and its chromatin granules become scattered throughout the body of the parasite. (Pl. VIII, figs. 8

[1] Lankester, Quart. Journ. Mic. Sci., n. ser., Vol. 11, 1871, p. 387.
[2] Zool. Jahrb., Abth. f. Anat., XV, 4, pp. 693–730, 1902.

and 9.) The sporont now becomes more spherical in shape, and surrounds itself with a membrane, thus being converted into an oocyst.

The chromatin granules of the fragmented nucleus now appropriate a certain quantity of the cyst-protoplasm, and then become sporoblasts, and these in turn are gradually changed into small rod-shaped bodies, or sporozoites (Pl. VIII, fig. 10), which when fully mature rupture the oocyst, and escape into the plasma (Pl. VIII, figs. 11 and 12), when they in turn will seek out and invade fresh blood corpuscles. Such I believe to be the sexual cycle of *Haemogregarina catesbianae,* as it now appears to me, but it is possible that after further study I may be forced to change my views.

Summary

Haemogregarina catesbianae is found in the blood of *Rana catesbiana* in several forms, among which may be mentioned, the merozoite or spore; the trophozoite, and cytocyst of the asexual cycle; the micro- and macrogametocytes, oocyst, and sporozoite of the sexual cycle, in which the microgametocyte is extra-corpuscular, while the macro-gametocyte is both extra- and intra-corpuscular.

Infection may be induced by the food taken into the animal's digestive tract, though this does not exclude infection from other causes.

Schizogony and sporogony occur in the red blood corpuscles of the same host.

In the asexual cycle, multiplication of the species is brought about by segmentation, or sporulation.

After many generations of schizogony, the sexes become differentiated into macro- and microgametocytes, and conjugate by some means yet undiscovered.

The extra-corpuscular macrogametocyte after fertilization, penetrates a red blood corpuscle, and becomes encysted, forming an oocyst. The chromatin granules of the fragmented cyst-nucleus appropriate a certain quantity of protoplasm, and become sporoblasts, which in turn are converted into germinal rods, or sporozoites, which when mature rupture the oocyst, and escape into the plasma, when they in turn are ready to invade fresh blood corpuscles.

EXPLANATION OF PLATES

Plate VII

Asexual cycle of *Haemogregarina catesbianae.* Magnification about 930 diameters.

Plate VIII

Sexual cycle of *Haemogregarina catesbianae.* Magnification about 930 diameters.

PLATE VII

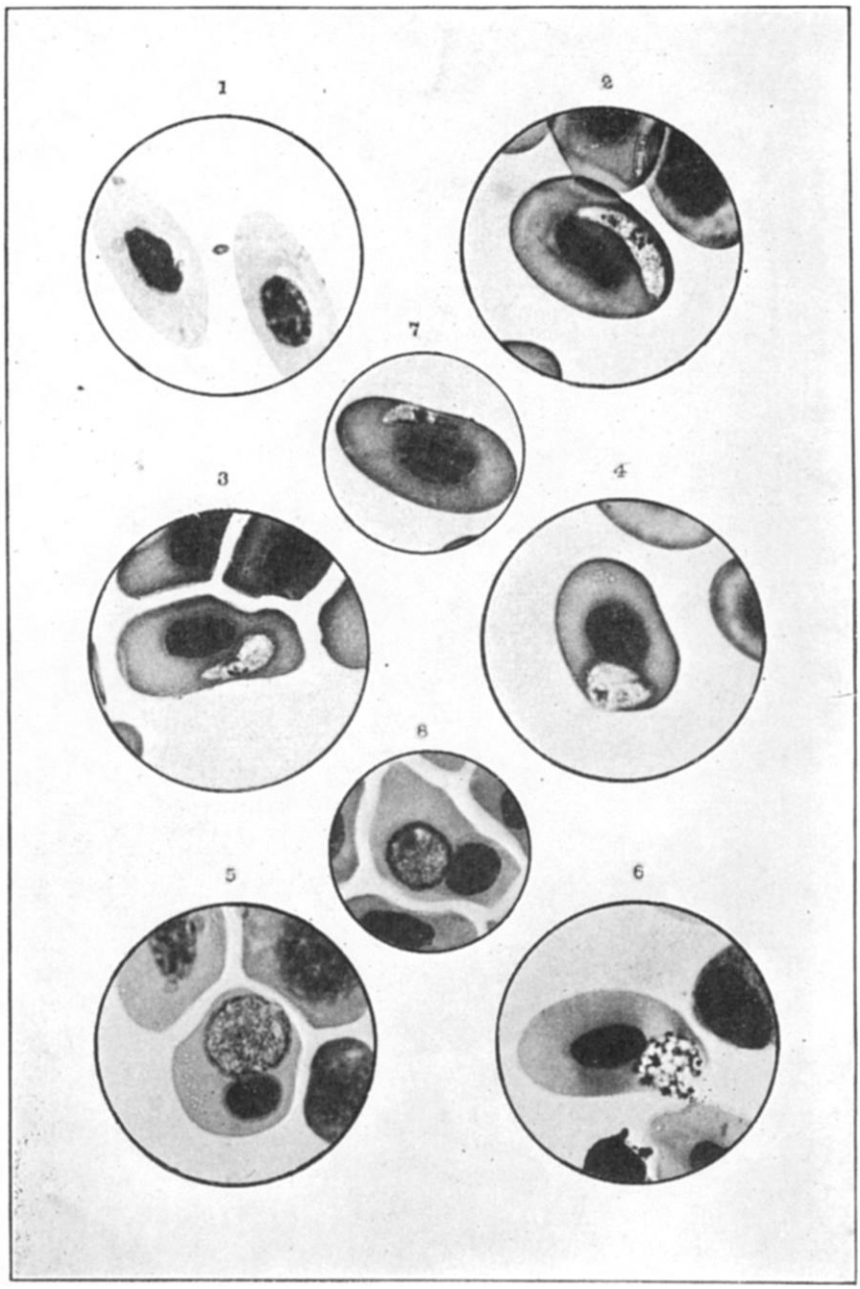

PLATE VIII